ADENOVIRAL VECTORS

CROSS-REFERENCE

[0001] This application is a 371 U.S. national phase of international application no. PCT/GB2018/052083, filed Jul. 24, 2018, which claims priority from GB patent application no. 1711971.0 filed Jul. 25, 2017 and GB patent application no. 1806375.0, filed Apr. 19, 2018, which applications are incorporated by reference in its entirety.

FIELD OF INVENTION

[0002] The present invention relates to an adenoviral vector comprising a regulatable Major Late Promoter and an exogenous transgene. The invention also provides cells comprising such adenoviral vectors, and processes using such vectors.

[0003] Adenoviruses are an attractive and versatile tool in biotechnology, having well understood genetics and the ability to grow to high yields in tissue culture. The replication cycle of Adenoviruses is highly complex, involving both early and late phases. The transition from early to late is considered to occur following DNA replication and the activation of the Major Late promoter (MLP) in the virus genome. The MLP drives the expression of all virus late transcripts, and can convert up to 30% of the cells' protein into virus structural proteins. Modification to the MLP in situ to provide inducible expression has not previously been demonstrated, primarily because the virus DNA polymerase coding sequence is in the opposing DNA strand.

[0004] Whilst adenoviruses are useful laboratory tools for a number of applications, the fact they are so productive represents a major problem if they are used in a manufacturing work flow, namely they generate substantial amounts of virus particles that must be removed during downstream processing. For example, when adenovirus has previously been used for protein expression purposes, or the production of other virus-like particles, or the large scale manufacture of Adeno-associated virus (AAV), the presence of intact Adenoviral particles at the end of the production process is highly undesirable.

[0005] The ability to manufacture proteins in mammalian cells is increasingly attractive, with many high value recombinant proteins now being produced in these systems, affording optimum protein processing, folding and glycosylation. This is frequently achieved using transient transfection of a plasmid encoding the required transgene under the control of a strong promoter, such as the Cytomegalovirus (CMV) immediate early promoter. However, compared to some viral systems in other organisms (such as Baculovirus in insect cells), this process is relatively inefficient, often demonstrating variable protein yields that rarely exceed 3% of the total cell protein mass.

[0006] The extensive characterisation of the Adenovirus genome, coupled with the wealth of knowledge regarding its gene expression and life cycle, makes this virus an ideal candidate platform on which to significantly improve recombinant protein yields. One major benefit is demonstrated by the virus' ability to actively hijack the mammalian cell's machinery, and to inhibit the production of cellular proteins.

[0007] However, in addition to inhibiting the production of cellular proteins, considerable cellular resources are used by the virus to produce viral structural proteins. The quantity of capsid proteins produced, for example, is vast and has been calculated to be up to 30% of total cell protein, and removing these proteins and the assembled virus particles after production is challenging.

[0008] Similar to recombinant proteins, the demand for adeno-associated virus particles is increasing significantly, owing to recent clinal successes in the treatment of retinal disorders and haemophilia. Traditionally, the production of AAV has been achieved through two different routes. Initially, AAV was generated using wild-type (WT) Adenovirus serotype 5 whilst transfecting cells with plasmids encoding the Rep and Cap genes and the AAV genome. This allowed the WT adenovirus to provide a number of factors in trans that facilitated virus replication. However, there a number of limitations to this approach: for example, each batch of AAV must be separated from the Ad5 particles to provide a pure product and ensuring that all Ad5 has been removed is challenging. Moreover, the fact that during production the cell is devoting huge resource to the production of Adenoviral particles rather than AAV is also undesirable.

[0009] More recently, the adenovirus-based systems have been replaced with plasmids encoding the sections of the Adenovirus genome required for AAV production. Whilst this has solved some of the concerns over Adenovirus particles being present in the final virus preparation, a number of issues remain. These include the requirement to pre-manufacture sufficient plasmid for transfection into the production cell line and the inherently inefficient process of transfection itself. The yields from these systems are also lower than those using Ad5 based approaches.

SUMMARY OF INVENTION

[0010] The inventors have now discovered that transcription of the late adenoviral genes can be regulated (e.g. inhibited) by the insertion of a repressor element into the Major Late Promoter. By "switching off" expression of the Late genes, the cell's protein-manufacturing capabilities can be diverted toward the production of a desired recombinant protein. Importantly, the strategic silencing of the Major Late promoter in the adenovirus genome in accordance with the current invention allows the virus to still replicate its DNA in the cell, providing thousands of DNA copies of the virus genome that can be transcribed for a range of applications.

[0011] This invention provides a range of advantages. For example, it can be used to direct a cell's protein production capability towards the production of specific recombinant proteins at increased yields compared to comparable systems wherein the viral Late genes are still expressed. Furthermore, the ability to "switch off" the production of viral structural proteins means that no or essentially no viral particles are produced during the protein-production process. Consequently, economic savings can be made due to a reduction in the need to remove virus particles from the purified protein.

[0012] The invention also has the advantage of providing a simple, cost-effective, way to manufacture AAV particles where the Rep and Cap proteins of AAV can be encoded within the Adenovirus to provide the high expression levels which are required to make the AAV particles by maintaining the replication of the Adenoviral genome, but also preventing the production Adenovirus particles in the final AAV preparation.

[0013] Some modifications of the MPL have previously been reported. These include making a copy of the MLP and